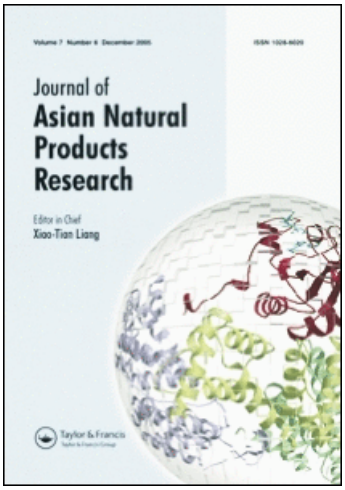

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Two new Daphniphyllum alkaloids from *Daphniphyllum macropodum* Miq.

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Two new Daphniphyllum alkaloids, 4,21-deacetyl-deoxyyuzurimine (**1**) and macropodumine L (**2**), together with the two known related alkaloids, have been isolated from the bark of *Daphniphyllum macropodum* Miq. The structures of the new compounds were elucidated on the basis of the detailed analysis of spectroscopic data, chemical method, and by comparison of the spectroscopic data with those of known compounds. Compounds **2** and **4** exhibited weak cytotoxicity against human carcinoma cell lines SMMC-7721 and HO-8910.

Keywords: *Daphniphyllum macropodum* Miq.; alkaloids; cytotoxicity

1. Introduction

Daphniphyllum alkaloids, elaborated by the trees of genus *Daphniphyllum*, are characterized by structurally diversified complex polycyclic skeletons [1], and have attracted great interest from a biogenetic [2] and synthetic [3] point of view. Recently, a series of novel Daphniphyllum alkaloid have been reported by Kobayashi [4], Hao [5], Yue [6], and our group [7], which have greatly widened the knowledge of this fascinating group of natural products.

Daphniphyllum macropodum Miq. is an evergreen tree that is widely distributed in the southern part of China, and the extract from its leaves and fruits is used for the treatment of inflammation in China [8].

In our continuing search for bioactive metabolites from Chinese medicinal plants [9], some novel alkaloids, either possessing unprecedented carbon skeleton or uncommon structural features, have been isolated from two different collections of title plant (Guangxi and Sichuan) [7]. Further chemical investigation of the bark of the Sichuan sample resulted in the isolation of two minor new alkaloids, 4,21-deacetyl-deoxyyuzurimine (**1**) and macropodumine L (**2**), together with the two known structurally related alkaloids, deoxyyuzurimine (**3**) [10] and daphniglaucin G (**4**) [11] (Figure 1). In this paper, we describe the isolation, structural elucidation, and the cytotoxic activity evaluation of the compounds **1**–**4**.

2. Results and discussion

The usual work-up [7] of the $CHCl_3$-soluble materials of the 95% EtOH extract of the bark of *D. macropodum* yielded the new compounds **1** and **2**, as well as the known alkaloids **3** and **4**.

The known alkaloids **3** and **4** were readily identified as deoxyyuzurimine [10] and daphniglaucin G [11], respectively, by the careful analysis of their NMR spectral data and by the comparison of the NMR spectral data with those reported in the literature.

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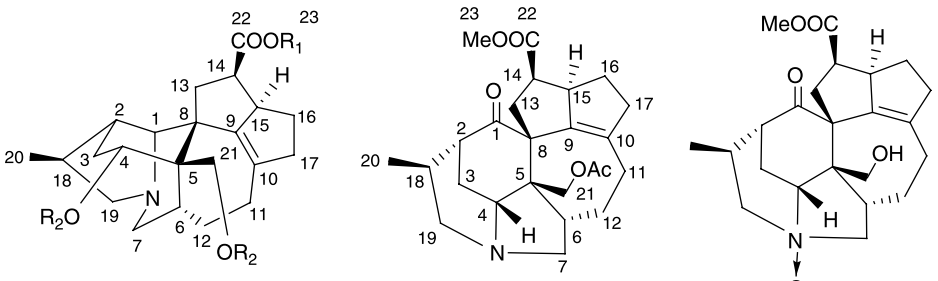

Figure 1. Structures of compounds **1**–**4**.

Compound **1**, 4,21-deacetyl-deoxyyuzurimine, was isolated as an optically active colorless oil ($[\alpha]_D^{24}$ − 20.4). Its molecular formula, $C_{23}H_{33}NO_4$, was determined by pseudo-molecular ion peak at *m/z* 410.2293 $[M + Na]^+$ in the HR-ESI-MS. The IR absorptions implied the presence of hydroxyl (3386 cm^{-1}) and ester (1732 cm^{-1}) functionalities. The ^{13}C NMR and DEPT spectra (Table 1) revealed signals due to one ester carbonyl, one tetrasubstituted double bond, two sp^3 quaternary carbons, seven sp^3 methines, nine sp^3 methylenes, and two methyls. One ester carbonyl and one double bond accounted for two degrees of unsaturation; thus, the remaining six degrees of unsaturation were ascribed conclusively to a hexacyclic ring system in compound **1**. Detailed analysis of the HSQC and $^1H—^1H$ COSY spectra revealed the presence of three partial structures: **a** (C-1 to C-4, C-2 to C-18, and C-18 to C-19 and C-20), **b** (C-6 to C-7 and C-12, and C-11 to C-12), and **c** (C-13 to C-17) (Figure 2). These subunits were linked together based on the HMBC correlations as shown in Figures 1 and 2. Careful comparison of the NMR spectral data of **1** and those of the co-occurring known alkaloid, deoxyyuzurimine (**3**), revealed that the former differs from the latter only for the absence of the two acetyl groups at C-4 and C-21, respectively, which was supported by the marked upfield shifted δ value of H-4 (from δ 5.30 in **3** to 4.32 in **1**) and H_2-21 (from δ 4.38 and 4.29 in **3** to 4.24 and 3.88 in **1**). The ^3J values (12.0 and 6.6 Hz between H-4 and H_2-3) suggested that H-4 took a β-axial orientation, which was further confirmed by the ROESY correlation observed between H-4 and H-13β (Figure 2). The relative configurations at the other chiral centers (C-1, C-2, C-5, C-6, C-8, C-14, C-15, and C-18) of **1**, identical to those of **3**, were established by ROESY cross-peaks of H_3-20/H-2 and H_2-3, H-4/H-21a, H-13β/H-21a, H-6/H-21b, H-4/H-13α, and H-15. Moreover, alkaline hydrolysis of **3** with 3% NaOH in MeOH afforded the expected deacetyl derivative exhibiting spectral data and the $[\alpha]_D$ value identical to those of **1**. Thus, the structure of **1** was unambiguously elucidated to be 4,21-deacetyl-deoxyyuzurimine.

It may be worth pointing out that compound **1** had been previously obtained as a synthetic intermediate in the chemical transformation from yuzurimine to yuzurimine B [12]. However, no NMR spectral data were reported in the literature. In this study, we have made the full ^1H and ^{13}C NMR assignments for this isolate (Table 1).

Compound **2** was shown to have the molecular formula of $C_{25}H_{33}NO_5$ by HR-ESI-MS. The ^{13}C NMR and DEPT spectra (Table 1) revealed 25 carbon signals due to one ketone carbonyl, two ester carbonyls, one tetrasubstituted double bond, two sp^3 quaternary carbons, six sp^3 methines, nine sp^2 methylenes, and three methyls. The ketone carbonyl carbon resonating at δ 215.2 was reminiscent of a carbon skeleton as that of

Table 1. ^{1}H and ^{13}C NMR spectral data[a,b] of compounds **1** and **2**.

	1[b]		**2**[b]	
Atom	δ_H (mult., *J*, Hz)	δ_C	δ_H (mult., *J*, Hz)	δ_C
1	2.60 (d, 3.1)	67.9 (d)	–	215.2 (s)
2	2.22 (m)	37.7 (d)	2.16 (m)	44.3 (d)
3α	1.51 (m)	31.4 (t)	2.05 (m)	20.7 (t)
3β	1.93 (m)	–	2.11 (m)	–
4	4.32 (dd, 12.0, 6.6)	72.9 (d)	3.52 (d, 4.5)	61.6 (d)
5	–	42.4 (s)	–	61.0 (s)
6	2.65 (m)	32.6 (d)	2.57 (m)	46.0 (d)
7a	3.22 (d, 12.9)	58.3 (t)	2.98 (m)	57.3 (t)
7b	3.05 (dd, 12.4, 9.2)	–	2.66 (dd, 9.0, 6.5)	–
8	–	45.9 (s)	–	54.3 (s)
9	–	144.4 (s)	–	140.7 (s)
10	–	134.9 (s)	–	139.3 (s)
11a	2.48 (m)	25.9 (t)	2.02 (m)	26.2 (t)
11b	2.04 (m)	–	2.02 (m)	–
12a	1.90 (m)	27.6 (t)	1.92 (m)	28.1 (t)
12b	1.50 (m)	–	1.72 (m)	–
13α	1.85 (m)	38.9 (t)	2.22 (dd, 14.0, 8.0)	39.4 (t)
13β	2.89 (m)	–	2.85 (t, 6.9)	–
14	2.94 (m)	43.5 (d)	2.74 (m)	41.7 (d)
15	3.52 (m)	54.7 (d)	3.42 (m)	53.3 (d)
16α	1.95 (m)	28.2 (t)	1.91 (m)	28.0 (t)
16β	1.18 (m)	–	1.25 (m)	–
17α	2.75 (m)	43.2 (t)	2.66 (m)	41.4 (t)
17β	2.38 (m)	–	2.35 (dd, 15.0, 9.0)	–
18	2.38 (m)	37.8 (d)	2.80 (m)	33.3 (d)
19a	3.51 (m)	65.1 (t)	2.79 (m)	49.8 (t)
19b	2.28 (m)	–	2.50 (m)	–
20	1.09 (d, 6.6)	15.9 (q)	1.00 (d, 6.5)	18.4 (q)
21a	4.24 (d, 11.6)	66.9 (t)	4.48 (d, 11.5)	67.2 (t)
21b	3.88 (d, 11.6)	–	4.28 (d, 11.5)	–
22	–	177.4 (s)	–	174.7 (s)
23	3.66 (s)	51.8 (q)	3.65 (s)	51.4 (q)
OAc-21			2.07 (s)	21.0 (q)
				170.9 (s)

[a] Recorded at 293 K on a Varian Mercury 400 MHz spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C NMR).
[b] Measured in $CDCl_3$, chemical shifts were referenced to the residual $CHCl_3$ (δ_H 7.26) for ^{1}H NMR, and to $CDCl_3$ (δ_C 77.0) for ^{13}C NMR.

daphniglaucin G (**4**). Careful comparison of the ^{13}C NMR spectral data of **2** and **4** revealed that the structure of **2** was similar to that of **4**. The main difference was that compound **2** exhibited an additional acetyl group and the N-oxide moiety in **4** disappeared. Significant HMBC correlation of H_2-21 with ester carbonyl carbon (δ 170.9, OAc) and marked downfield shift of H_2-21 (from 3.81 and 4.00 in **4** to 4.28 and 4.48 in **2**) led to the location of the acetyl group at C-21. In addition, the upfield shifted ^{13}C NMR values of C-4 (from δ 80.3 to 61.6), C-7 (from δ 76.7 to 57.3), and C-19 (from δ 67.0 to 49.8) in **2** were in agreement with the disappearance of N-oxide moiety. The β-orientation of H-2, H-4, H-6, H_3-20, and H_2-21 were determined by the analysis of the ROESY correlations of H-2/H-4, H-4/H-6, H_2-21/H-4, H_2-21/H-6, H_2-21/H-13β, and H_3-20/H-2. The ROESY cross-peaks between H-14 and H-13α and H-15 suggested that H-14 and H-15 were

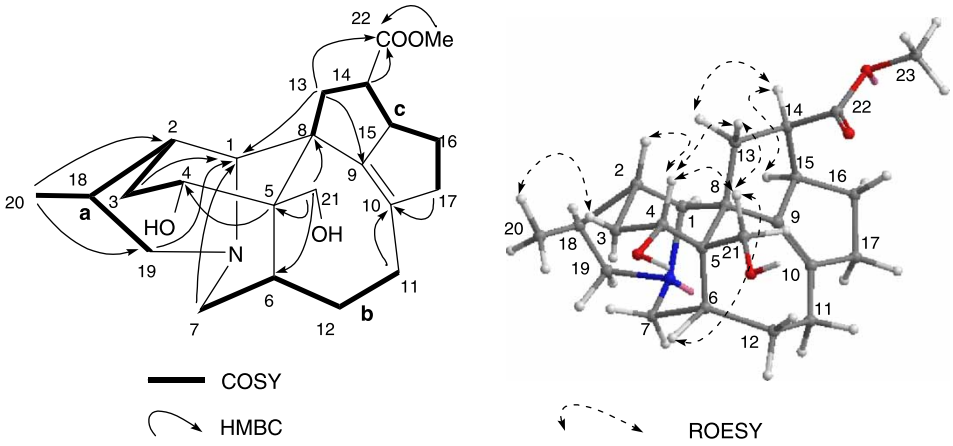

Figure 2. Selected 2D-NMR correlations of compound **1**.

α-orientated. Thus, the structure of **2** was depicted as shown in Figure 1, and named as macropodumine L.

Compounds **1**–**4** belonged to two different carbon skeletons. Although they are formally quite different, they are closely related biogenetically [13]. Loss of the leaving group at C-4 (the hydroxyl) by the attack of the nitrogen to form the N-1—C-4 bond will give daphniglaucin A. Further cleavage of the C-1—N bond will yield the carbon skeleton of compounds **2** and **4** (Figure 3).

Compounds **1**–**4** were tested for their cytotoxicities against a series of cancer cell lines, including human hepatocellular liver carcinoma cell line HepG2, human pancreatic adenocarcinoma cell line SW-1990, human lung cancer cell line NCI-H460, human breast adenocarcinoma cell line MCF-7, human ovarian carcinoma cell line HO-8910, and human carcinoma cell line SMMC-7721. The bioassay results indicated that compounds **2** and **4** exhibited weak cytotoxicity against human carcinoma cell line SMMC-7721 with the inhibition rate of 31 and 35%, respectively, at the concentration of 25 μg/ml. In addition, compounds **2** and **4** also showed weak inhibitory activity against human ovarian carcinoma cell line HO-8910 (inhibition rate 24 and 27%, respectively, 10 μg/ml).

Secondary metabolites represented adaptive characters that have been subjected to natural selection during evolution. They apparently function as defense (against herbivores, microbes, viruses, or competing plants) and signal compounds (to attract pollinating or seed dispersing animals) [14]. Further studies should be conducted on the biological/ecological role of these highly complex alkaloids in the life cycle of the plant from the evolutionary perspective.

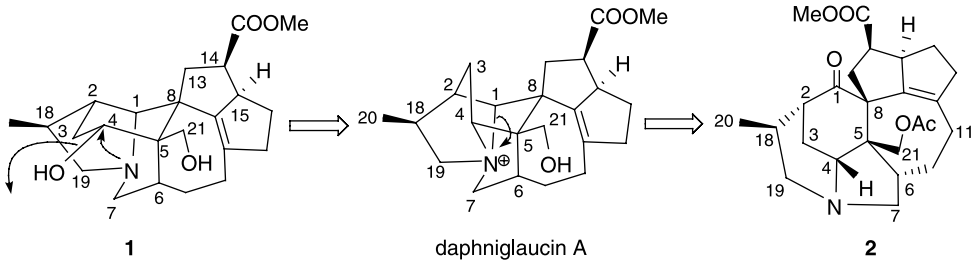

Figure 3. Plausible biogenetic relationship between **1** and **2**.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured on a Perkin-Elmer 341 polarimeter (PerkinElmer, Massachusetts, USA). The IR spectrum was recorded on a Nicolet Magna FT-IR 750 spectrometer (Thermo Scientific, Rockford, IL, USA). The ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury 400 (400 MHz for ^{1}H and 100 MHz for ^{13}C) spectrometer (Varian, Palo Alto, CA, USA). Chemical shifts (δ) were reported in parts per million with the residual $CHCl_3$ (δ_H 7.26) as the internal standards for ^{1}H, and $CDCl_3$ (δ_C 77.0) for ^{13}C NMR spectrometry, coupling constant (J) in Hertz. ESI-MS and HR-ESI-MS spectra were performed on a *Q-TOF Micro* LC–MS–MS mass spectrometer (Waters MS Technologies, Manchester, UK). Commercial Si gel (Qing Dao Hai Yang Chemical Group Co., 200–300 mesh, Qingdao, China), amino Si gel (Merck, LiChroprep NH_2 40–63 μm, Darmstadt, Germany), and Sephadex LH-20 (Amersham Biosciences, Little Chalfont, UK) were used for column chromatography. Precoated Si gel plates (Yan Tai Zi Fu Chemical Group Co., G60, F-254, Yantai, China) were used for analytical TLC.

3.2 Plant material

The plant specimen of *D. macropodum* Miq. was collected in the Emei Mountain, Sichuan Province of China, in April 2005, and identified by Prof. Hong-Gui Xu of Hong Kong Baptist University. A voucher specimen (P-17) is available for inspection at the Herbarium of the Institute of Matria Medica, Chinese Academy of Sciences.

3.3 Extraction and isolation

The air-dried powdered barks (1.4 kg) of *D. macropodum* were extracted with 95% EtOH (thrice, each 7 days) at room temperature. Evaporation of the solvent gave a residue, which was suspended in water (1 l) and adjusted to pH 4–5 with 2 N of H_2SO_4. The acidic mixture was defatted with EtOAc (3 × 1 l). The aqueous layer was basified to pH 9–10 with saturated Na_2CO_3 and then extracted with $CHCl_3$ (3 × 1 l) and *n*-BuOH (3 × 1 l). The $CHCl_3$ soluble material was subjected to Si gel column chromatography eluted with a $CHCl_3/CH_3OH/Et_2NH$ (50:1:0.1 to 1:1:0.1) gradient. The fractions eluted with $CHCl_3/CH_3OH/Et_2NH$ (25:1:0.1) were further purified by amino Si gel column chromatography to afford the alkaloids **1** (7.5 mg), **2** (8.7 mg), **3** (2310 mg), and **4** (5.3 mg).

3.3.1 4,21-Deacetyl-deoxyyuzurimine (*1*)

Colorless oil; $[\alpha]_D^{24}$ − 20.4 ($c = 0.53, CHCl_3$); IR ν_{max} (KBr) (cm^{-1}): 3386, 2921, 1732, 1436, 1195, and 1168; ^{1}H and ^{13}C NMR spectral data: see Table 1; ESI-MS: m/z 388 $[M + H]^+$ and 797 $[2M + Na]^+$; HR-ESI-MS: *m/z* 410.2293 $[M + Na]^+$ (calcd for $C_{23}H_{33}NO_4Na$, 410.2307).

3.3.2 Macropodumine L (*2*)

Colorless oil; $[\alpha]_D^{24}$ − 49.1 ($c = 0.61$, MeOH); IR ν_{max} (KBr) (cm^{-1}): 2923, 1736, 1436, 1236, 1195, and 1037; ^{1}H and ^{13}C NMR spectral data: see Table 1; ESI-MS: m/z 428 $[M + H]^+$ and 977 $[2M + Na]^+$; HR-ESI-MS: and *m/z* 428.2438 $[M + H]^+$ (calcd for $C_{25}H_{34}NO_5$, 428.2437).

3.3.3 Alkaline hydrolysis of compound *3*

A solution of 3% NaOH (0.5 ml) was added to a solution of **3** (10 mg) in MeOH (5 ml). The mixture was stirred at room temperature for 1 h and concentrated *in vacuo*. The residue was extracted with $CHCl_3$ and washed with water thrice, and further purified by Si gel column chromatography ($CHCl_3$/MeOH/Et_2NH: 40:1:0.1) to give the expected hydrolytic compound **1**.

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